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The Effects of Holothurin, a Steroid Saponin from the Sea Cucumber, on the Development of the Sea Urchin

GEORGE D. RUGGIERI, S. J., & ROSS F. NIGRELLI

Saint Louis University; Department of Marine Biochemistry and Ecology of the New York Aquarium, New York Zoological Society

(Plates I-IV)

INTRODUCTION

THE PURPOSE of the present study was to investigate the biological activity of the substance called Holothurin, a toxic metabolite from the Cuvierian organ of the Bahamian sea cucumber, *Actinopyga agassizi* Selenka, and to determine its mode of action. The effects of crude Holothurin on the development of the egg of the sea urchin, *Arbacia punctulata* (Lamarck), were compared with those of two fractions of the metabolite and with saponin. Findings were interpreted in the light of those of other chemical and physical agents already reported in the literature.

Arbacia punctulata is a relatively simple and accessible biological system that has frequently been used to elucidate the processes of morphogenesis. Characteristic abnormalities have been produced by means of changes in the physical environment, micrurgical techniques and diverse chemical agents. Lithium chloride (Herbst, 1892, 1893, 1896; Runnström, 1928; Child, 1940), antimetabolites (Gustafson & Horstadius, 1955), amino acids (Gustafson & Horstadius, 1957), bile salts (Lallier, 1954) and a host of other substances are known to modify the normal development of the sea urchin.

The principle which Nigrelli (1952) isolated and named Holothurin was the first known steroid saponin of animal origin. Nigrelli *et al.* (1955) and Chanley *et al.* (1955) have reported that Holothurin is highly soluble in water, nonvolatile and heat stable, exhibits surface-active properties, and appears to consist of a few steroid aglycones that are bound individually to four molecules of monosaccharides. Further purification of Holothurin and the analysis of its hydrolytic products by Chanley *et al.* (1959) supported the above view. Holothurin is thus placed in the class of cardiac glycosides or steroid saponins—substances previously found only in plants. Its saponin-like character, neurotoxic properties (Friess *et al.*, 1959) and hemolytic properties (Carson & Nigrelli, 1957) are all similar to those of drugs of plant origin. In addition, Holothurin contains one molecule of sulphuric acid, bound in ester linkage, which suggests a relationship to the steroid alcohols, *e.g.*, scymnol and ranol from the bile of the most primitive vertebrates. The glycosides of Holothurin resemble digitonin and other saponins in forming a complex with cholesterol. The monosaccharide components have been identified as glucose, xylose, glucomethylose (quinovose) and 3-0-methylglucose. The molecular weight of Holothurin was calculated to be 1155 and its composition $C_{50\text{-}52}$ $H_{81\text{-}85}$ $O_{25\text{-}26}$ SNa (Chanley *et al.*, 1959).

Studies carried out by Nigrelli and his asssociates have indicated that Holothurin is highly toxic to many types of organisms (Nigrelli, 1952; Nigrelli & Zahl, 1952; Nigrelli & Jakowska, 1958; Quaglio *et al.*, 1957). In addition to a neurotoxic effect, Holothurin has a direct contractural effect on muscle (Friess *et al.*, 1959). It also exhibits some anti-tumorous activity on Sarcoma 180 (Nigrelli, 1952; Nigrelli & Zahl, 1952) and on Krebs-2 ascites tumors in Swiss mice (Sullivan, Ladue & Nigrelli, 1955; Sullivan & Nigrelli, 1956), and in general shows a wide variety of pharmacodynamic actions, as is perhaps to be expected since it appears to be a complex of glycosides.

Materials and Methods

Three different solutions of Holothurin were tested on the sperm and various developmental stages of the egg of the sea urchin. Crude Holothurin is merely the sun-dried, powdered Cuvierian organ of the sea cucumber. The cholesterol-precipitated fraction, which is believed to be the pure active ingredient, has been designated as Holothurin A (Chanley *et al.,* 1959). The cholesterol-soluble fraction is here designated as Holothurin Fraction B. Purified saponin (Fisher Scientific Company) was used as a parallel control; this substance was used similarly in studying the hemolytic effect of Holothurin (Carson & Nigrelli, 1957).

Crude Holothurin, its two fractions, and saponin, are all readily soluble in sea water. Stock solutions (1/1,000) in sea water were freshly prepared for each series of experiments. Subsequent dilutions were made serially from the stock solution. No changes in pH were detected.

Adult sea urchins were collected at Far Rockaway Beach, New York, in mid-August. Males and females were maintained in separate 8-gallon aquaria, supplied with running sea water and fed occasionally with sea lettuce (*Ulva*). The sexes were determined by means of an electrical stimulation method, essentially the same as that reported by Harvey (1953), and the same method was used to obtain eggs and sperm. Using lead electrodes, an alternating current of 7 volts was applied to any two points on the aboral side of the test of the animal, which had been placed in a stacking bowl of sea water. The gametes were extruded from each of the gonopores almost immediately after the current was turned on; when the current was stopped the shedding ceased, to be resumed when the current was applied again. Approximately two dozen adults were used in the experiments.

Ripe eggs were washed in stacking bowls containing 200 cc. of sea water. Sperm was maintained undiluted until ready for insemination, at which time two drops (0.2 cc.) were diluted with 10 cc. of sea water. All collections of eggs and sperm were made at the time of an experimental run, so that neither eggs nor sperm remained standing for more than 10-15 minutes before insemination.

These experiments were performed from mid-August to December 2, 1958, at 23° C±1° C. Control experiments on December 2 showed the same high fertilizability (95-100%) as did earlier experiments, and development proceeded at the same rate and normally.

All experiments were performed in Syracuse dishes with the exception of one series in which stacking bowls were used. Between 25 and 50 eggs were placed in each dish containing 15 cc. of solution. This number was found to be well below the safe limit at which to avoid abnormatities from crowding. Evaporation was prevented by stacking the dishes.

In testing the action of Holothurin and its fractions on sperm motility, two separate small drops of diluted sperm were placed on a slide and observed through the Zeiss Lumipan microscope. An equivalent drop of the Holothurin solution, twice as strong as the desired final concentration, was mixed with one of the drops of diluted sperm, while an equivalent drop of sea water was mixed with the control drop. Motility of sperm in Holothurin was compared with that in sea water. In another series of experiments, undiluted sperm was exposed to various concentrations of Holothurin and its fractions for short periods of time and then allowed to inseminate untreated eggs.

Various developmental stages of the sea urchin egg were used as test animals: (1) unfertilized, (2) postfertilized (not more than 10-15 minutes after the addition of sperm), (3) 2-cell, (4) 4-cell, (5) 8-and 16-cell, (6) late cleavage (64-cell and beyond), (7) early blastula (at the appearance of first free-swimming blastulae, even though the majority were still unhatched, (8) mid-blastula (approximately two hours after first appearance of free-swimming blastulae, all now having hatched). These developmental stages were subjected to two types of treatment: (1) they were allowed to remain in test solutions throughout the remainder of their development; (2) they were treated for five minutes or less in test solutions and then returned to sea water. Controls in sea water were always maintained at the same time. Each series of experiments was performed on at least three different occasions. Since indistinguishable results were obtained on all occasions, the data have been consolidated in Tables I-XVII.

Unfertilized eggs were treated for five minutes in various test solutions and then returned to normal sea water where they were fertilized with untreated sperm. In the experiments where unfertilized eggs were treated "continuously," they were placed in various test solutions and after 15 minutes were inseminated by the addition of untreated sperm and left in the solutions throughout their development. All other stages were treated with the test solutions for five minutes and then returned to normal sea water, or were allowed to remain in the test solutions throughout their development.

Observations were made at predetermined intervals. Final, critical counts were not made before twelve hours after fertilization, at which time all controls consisted almost entirely of actively swimming forms. In order to achieve an objective measure of toxicity and a clear-cut distinction between those treated individuals that had suffered severe inhibition of development and those still definitely viable, those that were not free-swimming or moving freely within their membrane were considered to be "inhibited." The Zeiss Lumipan microscope, with apocromatic, phase and darkfield systems, and 10×, 20× and 40× objectives was used. All photographic work, unless otherwise indicated, was performed with this microscope and a Leitz camera attachment.

Following the terminology in the literature, the developmental modifications induced by Holothurin and its two fractions were characterized as:

1. Animalized larvae. (Figs. 2-4; 6-10). This form is characterized by an enlarged ciliary tuft and a thickened apical plate. Gastrulation fails to take place, and therefore the archenteron is lacking. Extreme cases produce, in 48-72 hours, uniformly ciliated blastulae of the type A of Horstadius (1939). Less extreme cases give rise to types B, C and D of Horstadius.

2. Fragmentation or "fragment balls." (Fig. 20). A fragment of the developing embryo breaks away and becomes free-swimming; it leaves behind a shell of cells and usually a partially dissolved membrane. Occasionally, similar but larger forms are observed swimming actively; these are accompanied by loose cells, lesser in number than those usually left behind by smaller "fragment balls."

3. Inhibition of hatching. (Fig. 19). Varying degrees of inability of the blastula to emerge from its membrane were noted: individuals that never hatch; those that are partially free of the membrane (*i. e.*, only a small portion of the membrane has been dissolved); those that are completely free of the membrane, but have left incompletely dissolved membranes as evidence of some difficulty in hatching.

4. Radially symmetrical larvae. (Figs. 11-15). These are elongated along their animal-vegetal axis. The apical ciliary tuft is enlarged and a band of short cilia surrounds the anal field. Varying degrees of archenteron formation are present. Spicules, if present, usually form a ring around the base of the archenteron. Some of these forms exhibit apical elongation, others present a greater hint at bilaterality.

5. Abnormally-formed plutei. (Figs. 16-18). These are bilateral and somewhat similar to control plutei. These larvae are usually characterized by a defective arrangment of the skeleton, typically an absence of arms, or short anal arms, or wide-angled arms. In some abnormal plutei, an over-development of the oral lobe was found. Larvae showing no abnormalities were classified as normal if they exhibited no deviation from the sea water controls. (Figs. 1, 5).

Observations and Results

With the exception of the experiments dealing with the treatment of sperm, the effects of the toxic compounds, crude Holothurin, Holothurin A and Holthurin Fraction B, were similar to and characterized by abnormal development not unlike that described in the literature for developing sea urchins subjected to various physical and chemical agents. Major differences within each series of experiments are concerned chiefly with the sequence, predominance and absence of one or more abnormalities.

Saponin produced the same results regardless of the stages treated (see Appendix, Tables I-XVI). With five-minute exposures to saponin, development was inhibited at a concentration of 1/1,000, but normal development ensued at a concentration of 1/10,000. With continuous exposures, saponin concentrates of 1/100,000 produced plutei the majority of which were normal. No animalized or radialized larvae were ever observed with saponin. An inhibition of hatching was observed in one experiment on 2-cell stages continuously exposed to 1/100,000 saponin. Dilutions greater than 1/100,000 saponin produced no noticeable effect upon development.

A. *Effects of Holothurin and its Fractions on Sperm*

Concentrations (final) of 1/20,000 and 1/200,000 crude Holothurin, Holothurin A and Holthurin Fraction B caused an immediate immobilization of sperm. Normal motility was apparent at concentrations of 1/2,000,000. High sperm activity was noted at the end of ten minutes, at which time this experiment was terminated.

The following observations were made when undiluted sperm was exposed for five minutes to various concentrations of crude Holothurin and its two fractions, and then allowed to fertilize untreated eggs: (1) no fertilization was observed after concentrations of 1/10,000 and 1/100,000; (2) exposure to 1/1,000,000 crude Holothurin and Holothurin A resulted in 50% of the eggs developing as in the sea water controls; a higher percentage (95%) of development, also indistinguishable from the controls, was observed with 1/1,000,000 Holothurin Fraction B;

(3) further dilutions of these three substances did not affect normal development.

Since treatment of the sperm was not effective in producing abnormal developmental patterns, all other experiments were concerned with treating the egg in various stages of development.

B. *Effects of Holothurin and its Fractions on Pre-fertilized Eggs*

1. Five-minute exposure (Table I).

Many of the eggs exposed to a concentration of 1/200,000 crude Holothurin and its two fractions were observed to develop to the morula stage, but no further. Those that developed further became animalized and were characterized by hyperciliation of the apical tuft and thickening of the apical ectoderm. After two or three days these forms became uniformly ciliated or presented a ciliated field or band.

A much higher percentage of development was observed with a concentration of 1/1,000,000. Both crude Holothurin and Holothurin A provoked predominantly animalized larvae, some radially symmetrical larvae and a few abnormal plutei. On the other hand, when unfertilized eggs were exposed for five minutes to Holothurin Fraction B, only a small number of radialized and animalized larvae were observed, the majority being abnormal and normal plutei.

At 1/10,000,000 no animalized larvae were observed, and radialized larvae were seen only with Holothurin A. Most of the larvae produced with this concentration of Holothurin and its fractions were normal and abnormal plutei.

Higher dilutions of crude Holothurin, Holothurin A and Holothurin Fraction B had no apparent effect on development.

2. Continuous exposure (Table IX).

From the results of the five-minute exposures, weaker concentrations with continuous exposures would be expected to elicit similar abnormalities.

In dilutions of more than 1/100,000,000, crude Holothurin and its two fractions had no effect on development; at this concentration, however, crude Holothurin and Holothurin A caused a large number of abnormal plutei, a lesser number of radialized larvae and a few animalized larvae. Eggs treated with Holothurin Fraction B developed normally in the great majority of cases and only a few abnormal plutei were formed.

The most striking effects were obtained when the eggs were treated with 1/10,000,000 crude Holothurin and its two fractions. Development of 85% to 90% was observed. Animalized larvae predominated in all three solutions. Some radialized larvae occurred in Holothurin Fraction B, and to a lesser degree abnormal plutei with all three solutions.

C. *Effects of Holothurin and Its Fractions on Various Developmental Stages of the Fertilized Egg*

1. Five-minute exposure (Tables II-VI).

The striking differences between the effects of Holothurin and its fractions on various stages of fertilized as compared with the unfertilized eggs is that inhibition of development occurred at weaker concentrations with the latter.

Concentrations of 1/100,000 crude Holothurin and Holothurin A always resulted in less than 50% development and produced either animalized larvae or "fragment balls." On the other hand, a higher percentage of development occurred with Holothurin Fraction B; these larvae were characterized by a predominance of animal types and a lesser number of abnormal plutei and "fragment balls."

Development of 75% to 95% resulted at 1/200,000 crude Holothurin and Holothurin A. These larvae were mostly animalized, some were radialized and a few were abnormal plutei. In contrast, Holothurin Fraction B produced primarily abnormal plutei with some radialized and animalized larvae and normal plutei. Some difficulty in hatching was observed in 2-cell eggs exposed to crude Holothurin.

A predominance of abnormal plutei prevailed when these stages were exposed for five minutes to 1/1,000,000 crude Holothurin and its fractions. A greater number of radialized and animalized larvae were observed in treatment with crude Holothurin and Holothurin A than with Holothurin Fraction B, while Holothurin Fraction B showed a greater number of normal plutei than did the other two substances. In addition, an inhibition of hatching was observed with Holothurin A in some of the tests on the post-fertilization, 2-cell and late cleavage stages. This phenomenon was also noted when 8- and 16-cell stages were exposed for five minutes to crude Holothurin, but Holothurin Fraction B exhibited this effect only with some 2-cell stages.

Development proceeded normally, for the most part, when these various early stages were exposed for five minutes to concentrations of 1/10,000,000 crude Holothurin and its fractions. Some abnormal plutei, more with crude Holothurin and Holothurin A than with Holothurin Fraction B, were also observed.

Greater dilutions of these substances had no apparent effect on the development of the egg of the sea urchin.

2. Continuous exposure (Tables X-XIV).

Development proceeded normally at concentrations of 1/1,000,000,000 crude Holothurin and its two fractions.

This same effect was observed with concentrations of 1/100,000,000 Holothurin Fraction B. The action of crude Holothurin and Holothurin A, on the other hand, was characterized by the development of a large number of abnormal plutei, some normal plutei, and a smaller number of radialized and animalized larvae.

At concentrations of 1/10,000,000 crude Holothurin and Holothurin A produced animalized and radialized larvae and a few abnormal plutei. The frequency of abnormalities was reversed in the case of Holothurin Fraction B, abnormal plutei being predominantly produced with lesser numbers of radialized and animalized larvae in addition to a few normal plutei. Difficulty in hatching was observed only with Holothurin A and this was limited to eggs immediately after fertilization and in the 2-cell stage.

Little or no development occurred in concentrations of 1/1,000,000 crude Holothurin and its fractions. The eggs that did develop became either "fragment balls" or animalized larvae.

D. *Effects of Holothurin and Its Fractions on Early and Mid-Blastula Stages*

1. Five-minute exposure (Tables VII and VIII).

Some difficulty in hatching was observed when early blastulae were exposed to concentrations of 1/100,000 to 1/1000,000 crude Holothurin and its fractions.

At concentrations of 1/100,000, Holothurin A was found to be most toxic; 10-35% development ensued, and only "fragment balls" and animalized larvae resulted. Approximately 50% development occurred with crude Holothurin; most of these became animalized and radialized larvae with a lesser number of "fragment balls" and abnormal plutei. In contrast, up to 70% development was observed in Holothurin Fraction B. All types of abnormalities were observed in this solution; the majority consisted of animalized and radialized larvae, with a lesser percentage of "fragment balls" and abnormal and normal plutei.

With the exception of "fragment balls," the same abnormalities described above for Holothurin Fraction B were observed when early blastulae were exposed for five minutes to a concentration of 1/200,000 crude Holothurin and its fractions.

A predominance of normal plutei, and lesser numbers of abnormal plutei and radialized and animalized larvae, occurred when early blastulae were exposed to concentrations of 1/1,000,000 crude Holothurin and its fractions. No animalized larvae were produced when mid-blastula stages were so treated; all the other above-mentioned forms, however, were found.

Development proceeded normally when mid-blastulae were exposed for five minutes to concentrations of 1/10,000,000 crude Holothurin and its fractions. This same effect was observed when early blastulae were exposed to this contration of Holothurin Fraction B. Although normal plutei prevailed when early blastulae were exposed to crude Holothurin and Holothurin A, some abnormal plutei were also observed, as well as a small number of radialized larvae with Holothurin A.

2. Continuous exposure (Tables XV and XVI).

Although no further development was observed when early and mid-blastula stages were continuously exposured to concentrations of 1/1,000,000 crude Holothurin and Holothurin A, 25% development did result in Holothurin Fraction B; all of the larvae became animalized.

No animalized larvae, however, were obtained when these stages were treated with 1/10,000,000 Holothurin Fraction B. Radially symmetrical larvae and some abnormal and normal plutei developed in Holothurin Fraction B, while a high percentage of animalized and radialized larvae developed in the same concentration of crude Holothurin and Holothurin A. Difficulty in hatching was noted only when early blastulae were treated with Holothurin A.

With the exception of some abnormal plutei, observed with the crude Holothurin and Holothurin A, a concentration of 1/100,000,000 Holothurin and its fractions produced plutei similiar to the controls. No effect on development was noted when blastula stages were continuously exposed to greater dilutions of the three substances.

E. *Additional Observations*

1. Five-minute exposure of 4-cell stages to Holothurin and its fractions, using 200 cc. in fingerbowls.

This series of experiments was performed to show that the small area and volume of the Syracuse dish were not responsible for any of the observed developmental modifications. Concentrations of 1/200,000 crude Holothurin and Holothurin A resulted in a predominance of animalized larvae, and a lesser number of radially symmetrical larvae. Holothurin Fraction B, in concentrations of 1/100,000, produced a majority of animalized larvae and occasional "fragment balls."

2. Concentration and time (Table XVII).

Crude Holothurin solutions of 1/10,000 in-

hibited development when 4-cell stages were exposed for 30 seconds, 1 minute and 3 minutes. Some development (25%) did take place, however, when these stages were exposed for 3 minutes to a concentration of 1/100,000; these were predominantly animalized larvae, but "fragment balls" were occasionally seen. A higher percentage of development occurred with 1-minute and 30-second exposures. Although the majority of the developing forms were normal in the 30-second exposure to 1/100,000 crude Holothurin, 1-minute treatment resulted in animalized and radialized larvae with a lesser number of abnormal plutei. As might be expected, 30-second exposures of 4-cell stages to 1/1,000,000 crude Holothurin had no effect on development; the same was true for 1-minute treatments except for a small number of abnormal plutei. When such cleavage stages were treated for 3 minutes, however, this concentration of crude Holothurin resulted in a predominance of abnormal plutei, some radialized and animalized larvae and a few normal plutei.

A concentration of 1/10,000 Holothurin A inhibited development no matter how short the treatment. Decreasing percentages of development were observed when 4-cell stages were exposed for 30 seconds, 1 minute and 3 minutes to 1/100,000 Holothurin A. Thirty-second exposures produced animalized and radialized larvae, and a lesser number of abnormal plutei, while only animalized larvae and "fragment balls" were observed in the 1- and 3-minute exposures. No animalized larvae were obtained when these stages were exposed for 30 seconds and 1 minute to 1/1,000,000 Holothurin A; in fact, results of the 30-second treatment were indistinguishable from the sea water controls. Although the majority of the forms subjected to a 1-minute exposure resembled the controls, some abnormal plutei and radialized larvae were in evidence. In contrast, a 3-minute treatment with this concentration of Holothurin A was sufficient to provoke animalization, as well as produce some radialized larvae and abnormal plutei.

Unlike crude Holothurin and Holothurin A, Holothurin Fraction B in a concentration of 1/10,000 produced a high percentage of animalized larvae when 4-cell stages were exposed for 30-seconds. As with crude Holothurin and Holothurin A, however, 1- and 3-minute exposures inhibited development. A 3-minute exposure to concentrations of 1/100,000 Holothurin Fraction B produced animalized and radialized larvae, and a small number of abnormal plutei. Exposures of 30 seconds and 1 minute, however, resulted in many larvae that closely resembled the sea water controls, with some abnormal plutei and a few radialized larvae also present. A 1/1,000,000 Holothurin Fraction B solution had no effect on the development of 4-cell stages exposed for 30 seconds and 1 minute. Except for occasional abnormal plutei, this was also true for the 3-minute exposure.

Discussion

It is evident from these experiments that Holothurin is a powerful toxic agent. Development was arrested when various stages were exposed for as little as five minutes to concentrations of 1/10,000, and with continuous exposure a concentration one-tenth as strong has the same effect. Concentrations of 1/100,000 in 5-minute exposures and 1/1,000,000 in continuous exposures permitted some development; many of the resulting individuals were only "fragment balls." Different degrees of fragmentation were noted; these resulted in minute blastulae of varying sizes. Lesser degrees of this phenomenon were evidenced by nearly normal-sized, actively swimming forms that trailed a string of loose cells.

Nigrelli & Zahl (1952) attributed an "all or none" effect to the biological activity of Holothurin, showing that the difference in dosage between "no effect" and "complete killing" was very small. Organisms exposed to lethal concentrations for short periods of time and then returned to normal media showed no recovery. An irreversibility of action was likewise demonstrated on amphibian and mammalian nerves by Friess *et al.* (1959). The present study indicates that this toxic metabolite can act in other ways than "all or none." A selective modification has been demonstrated on the developmental processes of the sea urchin, but this does not preclude an "all or none" effect upon certain ultracytoplasmic structures. Indeed, present observations indicate that Holothurin may have an irreversible effect at this level. Perhaps an indication of this irreversibility of Holothurin on certain ultracytoplasmic structures is afforded by the short term (five minutes or less) exposures of early stages to this substance. It would appear that if such an effect were reversible, a regulation to normality would be much more in evidence when these short term exposures were returned to normal sea water.

Holothurin A, which is believed to be the active ingredient of crude Holothurin, was no more efficacious than crude Holothurin in eliciting the same types of abnormalities at corresponding concentrations. Both crude Holothurin and Holothurin A were more effective than Holothurin Fraction B; all three substances, however, produced the same types of abnormalities.

Holothurin and its fractions exercise effects upon sea urchin development not obtainable with saponin. This was especially evident in short term exposures where concentrations of saponin either completely inhibited further development or else produced larvae that did not differ from the sea water controls. Although occasional abnormal plutei were produced, no animalized or radialized larvae were ever observed when any stage was continuously exposed to saponin.

The action of Holothurin is characteristic of the classical picture of animalization, *i.e.*, hyperdevelopment of the ciliary tuft, thickening of the apical ectoderm, absence of archenteron and appearance of uniformly ciliated blastulae and forms with ciliated fields or bands. These effects were elicited with 5-minute exposure to 1/100,000 Holothurin Fraction B, and 1/100,000–1/200,000 crude Holothurin and Holothurin A, as well as with continuous exposure to 1/1,000,000–1/10,000,000 Holothurin Fraction B and 1/10,000,000–1/100,000,000 crude Holothurin and Holothurin A.

In discussing the animalization produced by a lack of potassium or sulphate ions, treatment with thiocyanate in hypotonic solution and brief treatment with concentrations of mercuric ions, Lindahl (1942) maintained that this process is the result of a partial or complete deficiency of vegetal principle. Holothurin, therefore, must exert its effects upon the vegetal pole region. Horstadius (1935) reported that the extension of the apical ciliary tuft indicates the intensity of the animalization. When animalization is very strong, the apical ciliary tuft covers as much as three-fourths of the surface of the blastula, the thickening of the apical ectoderm is accentuated and extends laterally and the archenteron does not form. In older larvae the ciliary covering is composed of short cilia that extend over the entire surface of the blastula. In less animalized larvae a ciliary field or ciliary band encircling the oral field is differentiated.

Lindahl (1936) attempted to account for vegetalization and animalization by characterizing the animal pole as a region of carbohydrate metabolism and the vegetal as one of protein metabolism. Animalization would result from a block to protein metabolism which would reduce the amount of vegetalizing substance formed, and therefore the intensity of the vegetalizing influence. The unmodified animalizing influence would then turn the development of a larger proportion of the egg toward an ectodermal direction.

Ranzi & Falkenheim (1937) and Tamini (1943) believe that animalizing substances owe their effects to their ability to disperse colloids, vegetalizing substances to precipitate them. Ranzi and his collaborators (Arosio, *et al.*, 1949; Ranzi & Citterio, 1954; Ranzi, 1957) studied the action of different animalizing agents on the viscosity of proteins. They attributed an essential role to the differences in stability of proteins during the course of embryonic determination. Animalization thus entails the destruction of protein structures, while vegetalizing agents, which stabilize protein structures, inhibit this process.

Lallier (1958) also attributed animalizing and vegetalizing effects to an ability to change the structure of proteins, either directly or indirectly. Direct action affects protein molecules already formed. Ranzi and his collaborators have shown that the most active agents profoundly modify the properties of fibrillar and globular proteins, thus exhibiting a direct action on protein structure. Haurowitz (1956) pointed out that protein molecules acquire their characteristic configuration in the course of their synthesis and that the introduction of different substances (ions, macromolecules, etc.) changes the characteristics of the milieu, and this could interfere with the mechanisms responsible for the configuration of proteins.

Since Holothurin is obviously a complex substance that may contain multiple toxic factors, and since it is a substance of high surface activity, an interpretation of the possible mechanism or mechanisms of action is undoubtedly complicated. It is not likely, however, that the animalizing effects elicited by Holothurin and its fractions can be attributable merely to the high surface activity of these substances. Saponin also exhibits high surface-active properties, yet no animalizing effects were observed. Horstadius & Gustafson (1954) found that 8-chloroxanthine, a surface-active agent as well as an antimetabolite of purines and nucleic acid, produced radialized larvae and also exhibited a slight animalizing effect. They believed that the animalizing effect may have resulted solely from the antimetabolic action of 8-chloroxanthine, the radialization of larvae being attributable to the surface-active property of the substance. In analyzing the animalization by bile salts, Lallier (1954) was unable to associate this effect with a group common to these salts and other animalizing agents (such as thiocyanate, iodide, etc.). He therefore attributed the animalization to a direct effect on the ultracytoplasmic structure. He maintained that the surface-active properties of bile salts can play a part in the disruption of cytoplasmic structures. Finally, Lallier's interpretation of the results obtained by Lindahl with $HgCl_2$ are pertinent. Lindahl

(1936) showed that a 6½-minute exposure of fertilized eggs of the sea urchin, *Paracentrotus lividus,* to 1/90,000 concentration of $HgCl_2$ was sufficient to provoke animalization. Because of the short treatment and the weak concentration used, Lallier (1956b) suggested that the fixative action of the mercuric ion had been restricted to certain functional groups. A prolonged treatment would have resulted in the fixation by mercury of all disposable functional groups and thus would have inhibited development entirely.

In the present experiments, exposures of five minutes to low concentrations (1/100,000–1/1,000,000) of Holothurin and its fractions resulted in animalization. In another series of experiments, 1/100,000 crude Holothurin provoked animalization after only 1-minute exposure, the same concentration of Holothurin A produced a similar effect after a 30-second exposure, and 1/10,000 Holothurin Fraction B at 30-second exposures resulted in a high percentage of animalized larvae. Stronger concentrations or longer exposures inhibited further development. This may indicate that at lower concentrations or shorter exposures the action of Holothurin and its fractions is limited to certain functional groups, and at stronger concentrations or longer exposures the action of Holothurin and its fractions effects a more widespread fixation, and thus prevents further development.

It appears that Holothurin may elicit its animalizing effects by modifying or destroying certain protein structures already formed in the developing sea urchin egg or by interfering with its synthesis of proteins. The possible action of Holothurin on protein structures was implied by Nigrelli & Zahl (1952), who reported that the toxicity of Holothurin to protozoa in synthetic media was lessened by the addition of complex natural materials such as mixtures of nucleic acids and protein hydrolysates. These substances could afford a protective mechanism by forming complexes with Holothurin, rendering it less available to combine with the proteins of the protozoa.

Since the complete chemical structure of Holothurin is not known, it is impossible to attribute its action to any specific chemical group or groups. However, some tentative considerations, based on what is already known about its chemistry, can be made.

Lallier (1955c, 1956a, 1957a, b) obtained animalizations using various sulfonic acid dyes and polysulfonic derivatives. He concluded that the presence of acidic groups appears essential for the manifestation of animalizing effects or the change in type of symmetry. These acidic groups can be sulfonic or carboxylic, and they presumably produce their effects by combining with the basic groups of proteins. Animalizing properties would also be influenced by the position of the acidic groups within the molecule, and by the presence of other groups. The latter could act either directly by combining with cellular constituents, or indirectly by augmenting the action of the acid. Important properties, such as solubility, penetrability and affinity for cellular constituents, depend upon the number and nature of the nuclei that comprise a chemical agent. According to Lallier, all these properties of the chemical agent play important roles in provoking animalization.

In Holothurin, the presence of a molecule of sulphuric acid, although bound in ester linkage, may bring about an effect similar to that of the sulfonic derivatives mentioned above. Indeed, one of the differences between Holothurin and saponin is the presence of sulphuric acid. However, the use of the fractions of Holothurin did not bring the possible function of the sulphuric acid group into as sharp a focus as was hoped. Holothurin A, the cholesterol-precipitated fraction, contains glycosides as well as sulphuric acid. Holothurin Fraction B, which is the cholesterol-soluble fraction, theoretically is devoid of both glycosides and sulphuric acid. Our tests, however, show that Holothurin Fraction B produces the same animalizing effects as Holothurin A and crude Holothurin, but in stronger concentrations. It is, of course, possible that the animalizing effect may be the result of some group or groups other than the sulphuric acid and/or glycoside. Another possibility is that the cholesterol-soluble fraction may contain some glycoside and/or sulphuric acid; this would account for the stronger concentration of Holothurin Fraction B needed to provoke animalization. The fact that Holothurin and its fractions are agents with high surface-active properties may play a vital role in the adherence and penetrance of these substances into the cell. The indication that the steroid portion of Holothurin is different from that of other saponins may likewise play an important role in eliciting these animalizing effects.

The production of radialized larvae has been reported by many workers, using diverse chemical agents. Gustafson & Savhagen (1950) found that anionic detergents, such as duodecylsulphate, laurylsulphate, "Duponol" and "Texapon" very efficiently induce radialization. Horstadius & Gustafson (1954) obtained the same effect using 8-chloroxanthine, an antimetabolite of purines and nucleic acid, and β-phenyllactic

acid, an antimetabolite of phenylalanine. Rulon (1952, 1953a, b, 1955, 1956, 1957) induced radialization in the sand dollar by treatment with glucose and the salts of zinc, nickel and cobalt. Lallier (1955a, b, c, 1956a, b, 1957b) observed radializing effects with such substances as zinc, various sulfonic derivatives and sodium persulphate.

The radialization produced by anionic detergents (Gustafson & Savhagen, 1950) has been interpreted by Horstadius & Gustafson (1954) as a result of the high surface activity of these substances. These authors believed that these compounds have no pronounced antimetabolic properties. They also indicated that the radializing effect produced by the antimetabolites, 8-chloroxanthine and β-phenyllactic acid, should be attributed to their surface-active properties. On the other hand, Lallier (1957b), in explaining the appearance of animalized larvae at stronger concentrations of a particular chemical agent and radialized larvae at weaker ones, regarded radialization as representing a weakened form of animalization.

The radialized larvae produced by the action of Holothurin pose certain problems. To attribute this effect to its surface-active properties alone appears to be an oversimplification. The complete lack of radialized larvae in the presence of saponin, also a substance of high surface activity, supports this view. Animalized larvae were obtained with the more concentrated solutions of Holothurin, while radialized larvae usually occurred in the less concentrated ones. As Lallier (1957b) sugggested, it appears likely that radialized larvae do represent a less extreme form of animalization.

There is, however, one effect that seems to be specifically the result of the high surface activity of crude Holothurin and its two fractions. Throughout this study there have been instances where Holothurin solutions had an effect on the hatching of the sea urchin blastula and the dissolution of the membrane. This was noted most frequently with Holothurin A, less with crude Holothurin and least with Holothurin Fraction B. It occurred in various degrees. At times blastulae were unable to hatch from their membranes and were observed rotating within their membranes. Other blastulae were only partly free of the membrane, or if free, left incompletely dissolved membranes on the bottom of the dish as evidence of some difficulty in hatching. This effect was also observed, though much less frequently, in some experiments with saponin. There is obviously some interference in the reaction between the hatching enzyme and the membrane (substrate). It does not appear likely that the enzyme is inactivated. It seems probable that a film forms on the membrane and prevents the action of the enzyme on its substrate. This explanation is the more plausible for several reasons: (1) the high surface activity of Holothurin and its fractions, which makes the formation of a film likely; (2) the presence of varying degrees of inhibition of hatching, and the lack of this phenomenon in similar experiments with Holothurin; and (3) the rare occurrence of inhibition of hatching with saponin, also a substance of high surface activity. A similar effect has been alluded to by Horstadius & Gustafson (1954) in attempting to explain the appearance of radially symmetrical larvae treated with 8-chloroxanthine and β-phenyllactic acid.

The appearance of abnormally formed plutei, observed with lower concentrations of crude Holothurin and its two fractions, both in short term and continuous exposures, may be a recovery reaction (Child, 1941). Such plutei were characterized by a defective skeleton, typically a complete absence of arms, short anal arms or wide-angled arms. There appears to have been disturbance, but the stimulus was not strong enough and/or did not persist long enough to produce forms more divergent from the controls than these abnormally formed plutei.

Summary

1. Crude Holothurin, a toxic steroid saponin from the sea cucumber, *Actinopyga agassizi*, and two of its fractions, Holothurin A and Holothurin Fraction B, were tested in various concentrations on the developmental stages of the sea urchin, *Arbacia punctulata*. Purified saponin was used as a parallel control in addition to the usual controls in sea water.

2. The Holothurin compounds were shown to cause developmental modifications in the sea urchin not obtainable with saponin. Holothurin and its fractions inhibited development at concentrations which produced no effects on the developmental processes when saponin was used.

3. The most significant modification produced by Holothurin and its two fractions was animalization, indicating that certain protein structures in the developing sea urchin were affected. Other modifications in developmental patterns were fragmentation, inhibition of hatching, radialized larvae and abnormally formed plutei.

4. Crude Holothurin and Holothurin A were effective in weaker concentrations (5-10×) than was Holothurin Fraction B. All three substances are extremely powerful agents, producing developmental modifications at extremely low concentrations and at very short exposures.

Appendix

Table I. Five-minute Exposure of Unfertilized Eggs to Crude Holothurin, Holothurin A, Holothurin Fraction B and Saponin*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	85% P
1/100,000	0	0	0	95% P
1/200,000	40% a	25% a	50% a	95% P
1/1,000,000	85% arp	85% arp	95% pPra	
1/10,000,000	95% Pp	95% Ppr	95% Pp	
1/20,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin Fraction B; a = animalized larvae; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

Table II. Five-minute Exposure of Eggs Immediately Post-fertilization to Crude Holothurin, Holothurin A, Holothurin Fraction B and Saponin*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	85% P
1/100,000	1-10% a	0	85% ar	95% P
1/200,000	85% ar	75% ar	95% praP	95% P
1/1,000,000	95% praP	95% rapPi	95% Pp	
1/10,000,000	95% Pp	95% Pp	95% P	
1/100,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

Table III. Five-minute Exposure of 2-cell Stages to Crude Holothurin, Holothurin A, Holothurin Fraction B and Saponin*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	95% P
1/100,000	50% af	30% af	85% arp	95% P
1/200,000	95% arpi	85% arp	95% parP	
1/1,000,000	95% praP	95% praPi	95% pPrai	
1/10,000,000	95% Pp	95% Pp	95% Pp	
1/100,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

Table IV. Five-minute Exposure of 4-cell Stages to Crude Holothurin, Holothurin A, Holothurin Fraction B and Saponin*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	95% P
1/100,000	30% af	10% af	50% af	95% P
1/200,000	85% ar	75% ar	95% arp	
1/1,000,000	95% rapP	95% arpP	95% pPra	
1/10,000,000	95% Pp	95% Pp	95% Pp	
1/100,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE V. FIVE-MINUTE EXPOSURE OF 8- AND 16-CELL STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	95% P
1/100,000	0-10% f	0-5% f	40% ar	95% P
1/200,000	75% ar	75% ar	95% rapP	
1/1,000,000	95% arpPi	95% arpP	95% pP	
1/10,000,000	95% Pp	95% Pp	95% P	
1/100,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE VI. FIVE-MINUTE EXPOSURE OF LATE CLEAVAGE STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	95% P
1/100,000	40% af	30% af	60% ar	95% P
1/200,000	85% arp	85% arp	95% pPra	
1/1,000,000	95% pPr	95% pPri	95% Pp	
1/10,000,000	95% Pp	95% Pp	95% P	
1/100,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE VII. FIVE-MINUTE EXPOSURE OF EARLY BLASTULA STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	95% P
1/100,000	50% afri	35% fai	70% afrpi	95% P
1/200,000	85% praPi	85% aprPi	95% praP	
1/1,000,000	95% Pprai	95% rPpai	95% Ppr	
1/10,000,000	95% Pp	95% Ppr	95% P	
1/100,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE VIII. FIVE-MINUTE EXPOSURE OF MIDBLASTULA STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/1,000	0	0	0	0
1/10,000	0	0	0	100% P
1/100,000	60% arp	10% fa	75% prP	100% P
1/1,000,000	95% Ppr	95% Ppr	95% Ppr	
1/10,000,000	100% P	100% P	100% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE IX. CONTINUOUS EXPOSURE OF PREFERTILIZED EGGS TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% pP
1/1,000,000	0	0	5-10% a	95% P
1/10,000,000	85% ap	85% ap	95% arp	95% P
1/100,000,000	95% prai	95% pra	95% Pp	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE XI. CONTINUOUS EXPOSURE OF 2-CELL STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% pPi
1/1,000,000	0	0	25% af	95% P
1/10,000,000	50% ar	50% ari	85% pra	95% P
1/100,000,000	95% pPra	95% pPra	95% Pp	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE X. CONTINUOUS EXPOSURE OF EGGS IMMEDIATELY POST-FERTILIZATION TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% Pp
1/1,000,000	0	0	0	95% P
1/10,000,000	50% a	50% ai	80% a	95% P
1/100,000,000	95% praP	95% praP	95% Ppra	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE XII. CONTINUOUS EXPOSURE OF 4-CELL STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% Pp
1/1,000,000	1-5% fa	0	30% a	95% P
1/10,000,000	85% rpa	85% rap	95% prP	95% P
1/100,000,000	95% pP	95% pP	95% P	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE XIII. CONTINUOUS EXPOSURE OF 8- AND 16-CELL STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% Pp
1/1,000,000	5% fa	0	30% a	95% P
1/10,000,000	85% rpa	85% rap	95% prP	95% P
1/100,000,000	95% pP	95% pP	95% P	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls;" r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE XIV. CONTINUOUS EXPOSURE OF LATE CLEAVAGE STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% Pp
1/1,000,000	5% af	0	10% af	95% P
1/10,000,000	85% par	85% a	95% Ppra	95% P
1/100,000,000	95% Pp	95% Ppr	95% P	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; f = "fragment balls"; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE XV. CONTINUOUS EXPOSURE OF EARLY BLASTULA STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% Pp
1/1,000,000	0	0	25% a	95% P
1/10,000,000	85% ar	85% ari	95% rpP	95% P
1/100,000,000	95% Pp	95% Pp	95% P	
1/1,000,000,000	95% P	95% P	95% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; r = radialized larvae; i = inhibition of hatching; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

TABLE XVI. CONTINUOUS EXPOSURE OF MID-BLASTULA STAGES TO CRUDE HOLOTHURIN, HOLOTHURIN A, HOLOTHURIN FRACTION B AND SAPONIN*

Percentage Developing and Types of Abnormalities Produced

Conc.	Crude	A	B	Saponin
1/10,000	0	0	0	0
1/100,000	0	0	0	85% Pp
1/1,000,000	0	0	25% a	100% P
1/10,000,000	85% ar	85% ar	95% rpP	100% P
1/100,000,000	95% Pp	95% Pp	100% P	
1/1,000,000,000	100% P	100% P	100% P	

*Crude = Crude Holothurin; A = Holothurin A; B = Holothurin fraction B; a = animalized larvae; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

Table XVII. Concentration and Time (with 4-cell Stages)*

Percentage Developing and Types of Abnormalities Produced

Crude Holothurin	30 sec.	1 min.	3 min.
1/10,000	0	0	0
1/100,000	95% Pp	50% arp	25% af
1/1,000,000	95% P	95% Pp	95% praP
Holothurin A	**30 sec.**	**1 min.**	**3 min.**
1/10,000	0	0	0
1/100,000	50% arp	30% af	10% af
1/1,000,000	95% P	95% Ppr	95% arp
Holothurin Fraction B	**30 sec.**	**1 min.**	**3 min.**
1/10,000	50% a	0	0
1/100,000	95% Ppr	95% Ppr	75% arp
1/1,000,000	95% P	95% P	95% Pp

*a = animalized larvae; f = "fragment balls"; r = radialized larvae; p = abnormal plutei; P = plutei similar to control; listed according to frequency.

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EXPLANATION OF THE PLATES

All figures are photomicrographs of living stages

PLATE I

FIG. 1. Control blastula. Note extension of apical ciliary tuft. Darkfield illumination. Approximately 100×.

FIG. 2. Animalized larva—24 hours. Hyperciliation of the apical ciliary tuft. Darkfield illumination. Approx. 200×.

FIG. 3. Animalized larva—24 hours. Hyperciliation of the apical ciliary tuft. Phase contrast. Approx. 400×.

FIG 4. Animalized larva—48 hours. Note thickening of apical ectoderm. Phase contrast. Approx. 200×.

PLATE II

FIG. 5. Control plutei—2-3 days. Phase contrast. Approx. 100×.

FIG. 6. Animalized larva—72 hours. Uniformly ciliated blastula. Phase contrast. Approx. 400×.

FIG. 7. Animalized larva—72 hours. Blastula with ciliated field. Phase contrast. Approx. 400×.

FIG. 8. Animalized larva—72 hours. Blastula with ciliated band. Phase contrast. Approx. 400×.

FIG. 9. Animalized larva—72 hours. Blastula with ciliated band. Phase contrast. Approx. 200×.

FIG. 10. Animalized larva—72 hours. Blastula with ciliated band. Phase contrast. Approx. 200×.

PLATE III

FIG. 11. Radialized larva—72 hours. Note cilia surrounding anal field. Phase contrast. Approx. 400×.

FIG. 12. Radialized larva—72 hours. Apical elongation. Phase contrast. Approx. 200×.

FIG. 13. Radialized larva—72 hours. Note archenteron. Phase contrast. Approx. 400×.

FIG. 14. Radialized larva—72 hours. Note cilia. Phase contrast. Approx. 200×.

FIG. 15. Radialized larva—72 hours. Note skeletal spicules. Phase contrast. Approx. 100×

PLATE IV

FIG. 16. Abnormal pluteus. Absence of arms. Phase contrast. Approx. 400×.

FIG. 17. Abnormal pluteus. Defective skeletal arrangement. Phase contrast. Approx.. 200×.

FIG. 18. Abnormal plutei. Note wide-angled arms of plutei. Phase contrast. Approx. 100×.

FIG. 19. Inhibition of hatching. Note incompletely dissolved membranes by which young blastulae are held together. Phase contrast. Approx. 100×.

FIG. 20. Fragmentation. Small embryonic core emerges as free-swimming. Phase contrast. Approx. 100×.

FIG. 1

FIG. 2

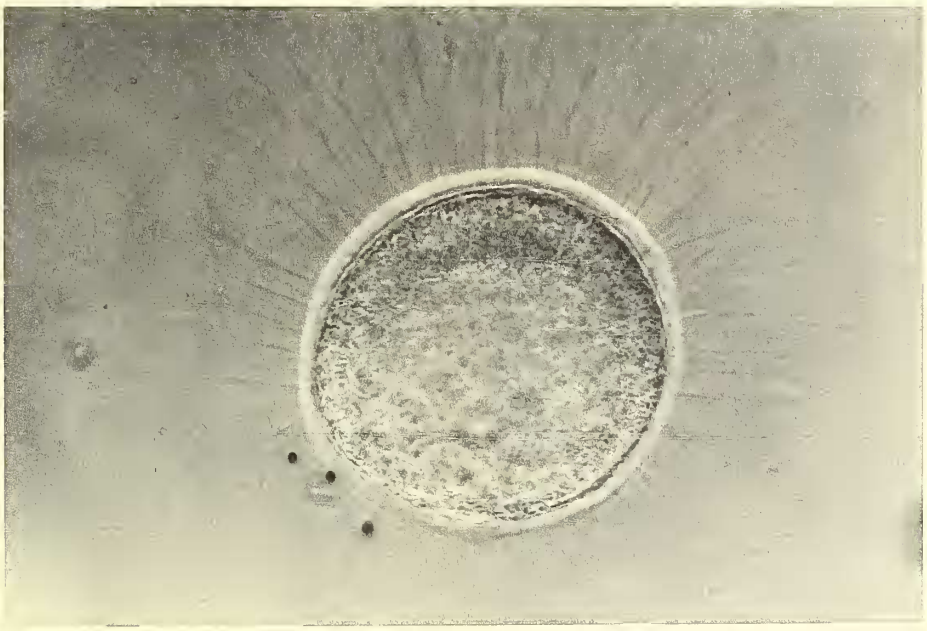


FIG. 3

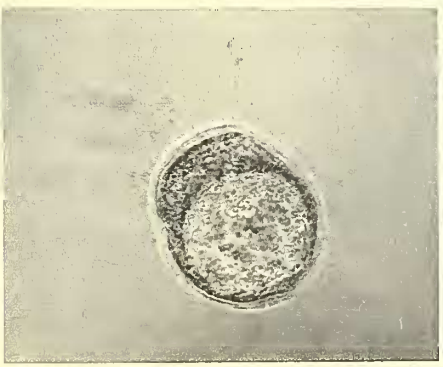


FIG. 4

THE EFFECTS OF HOLOTHURIN, A STEROID SAPONIN FROM THE SEA CUCUMBER, ON THE DEVELOPMENT OF THE SEA URCHIN

FIG. 5

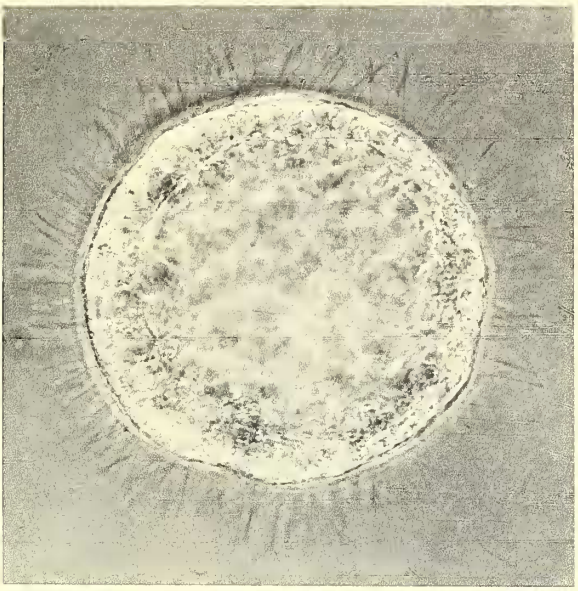
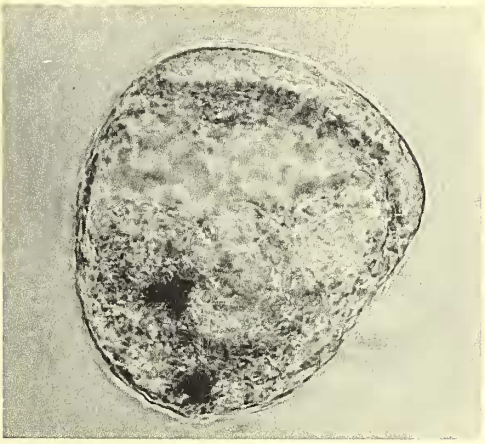


FIG. 6



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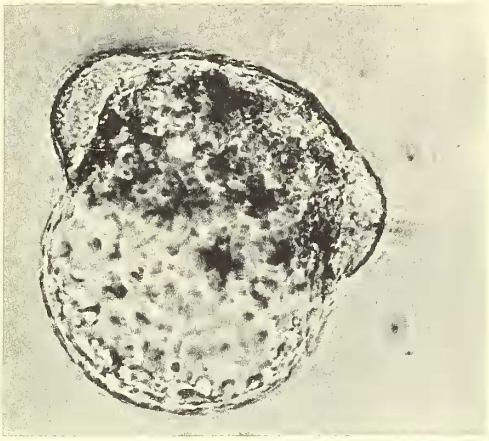


FIG. 8

FIG. 9

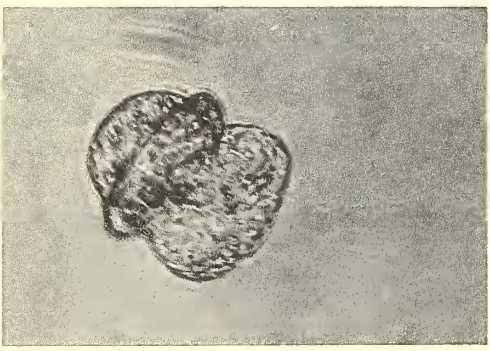


FIG. 10

THE EFFECTS OF HOLOTHURIN, A STEROID SAPONIN FROM
THE SEA CUCUMBER, ON THE DEVELOPMENT OF THE SEA URCHIN

FIG. 11

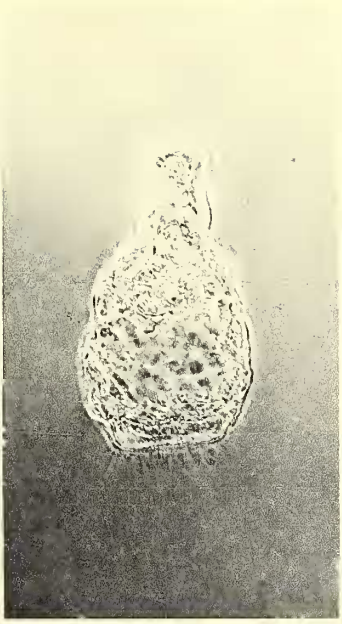


FIG. 12

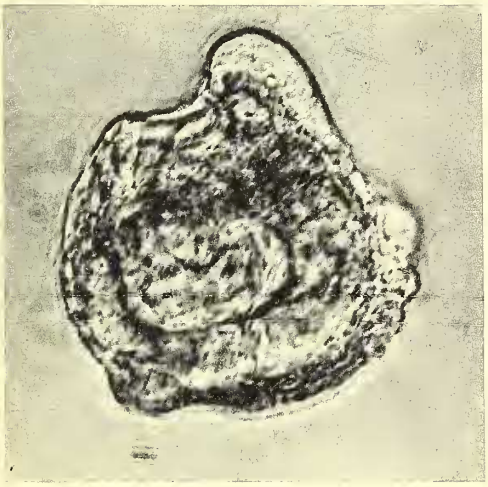
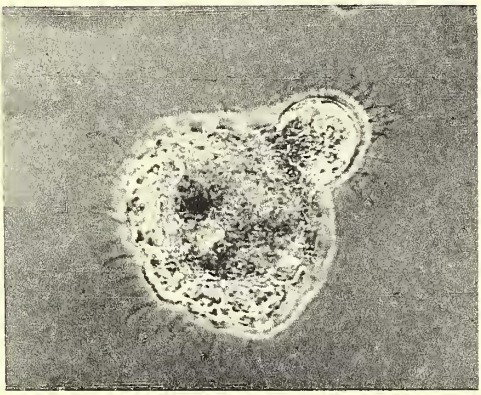


FIG. 13



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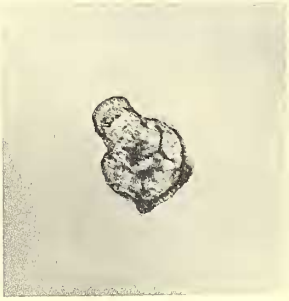

FIG. 15

THE EFFECTS OF HOLOTHURIN, A STEROID SAPONIN FROM
THE SEA CUCUMBER, ON THE DEVELOPMENT OF THE SEA URCHIN

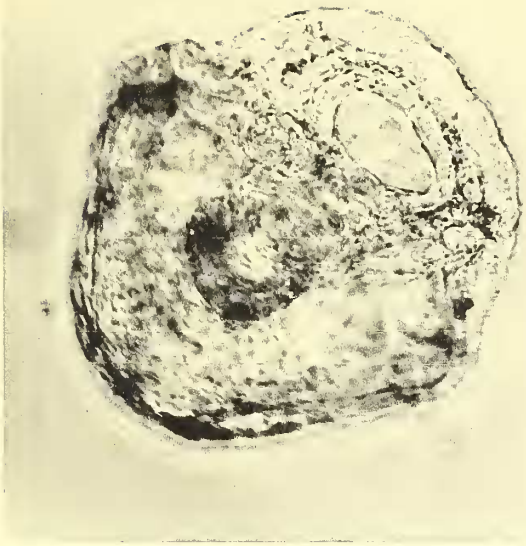

FIG. 16

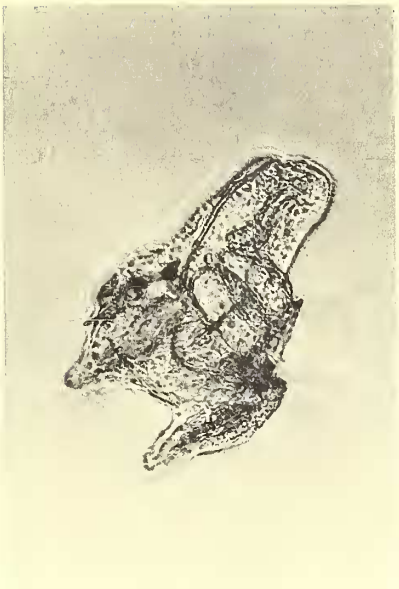

FIG. 17

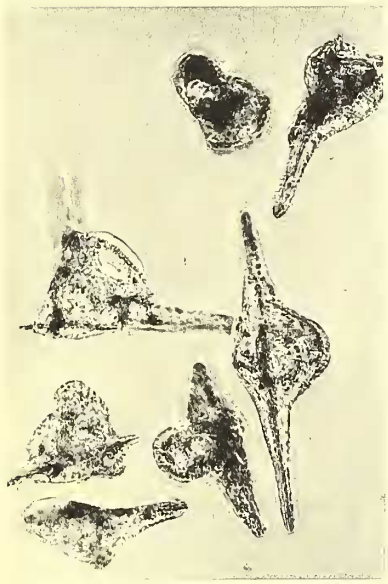

FIG. 18

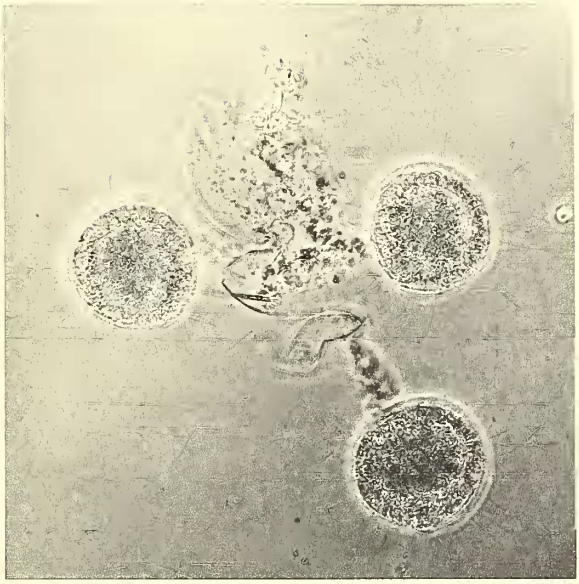

FIG. 19

FIG. 20

THE EFFECTS OF HOLOTHURIN, A STEROID SAPONIN FROM
THE SEA CUCUMBER, ON THE DEVELOPMENT OF THE SEA URCHIN